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DETERMINATION OF 1,1-DIMETHYLHYDRAZINE (UDMH) IN URINE

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FOREWORD

This work was performed under Project No. 6302, "Toxic Hazards of Propellants and Materials," Task No. 630202, "Pharmacology and Biochemistry," from October 1960 to July 1962 in the Toxic Hazards Section, Physiology Branch, Biomedical Laboratory, 6570th Aerospace Medical Research Laboratories. The valuable assistance and suggestions of Mildred K. Pinkerton are gratefully acknowledged.

The experiments reported herein were conducted according to the "Principles of Laboratory Animal Care" established by the National Society for Medical Research.

ABSTRACT

The colorimetric determination of 1, 1-dimethylhydrazine (UDMH) in urine using trisodium pentacyanoamino ferroate (TPF) has heretofore been hampered by interfering substances in urine which resulted in poor recovery and some false negative results. A procedure was developed which overcame this difficulty and was primarily designed to determine rapidly in an emergency situation the urinary concentration of UDMH in exposed personnel. The procedure involves a hydrogen peroxide treatment of the urine followed by a colorimetric complexing of UDMH with TPF. In vivo results are presented on dogs that received intraperitoneal doses of UDMH with urinary concentrations of UDMH being determined in spontaneously voided and 24-hour pooled specimens. The results give some indication of the relationship of dose to excretion levels. In vitro recovery in 100 human urine specimens is presented.

PUBLICATION REVIEW

This technical documentary report has been reviewed and is approved.

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INTRODUCTION

Large-scale use of 1,1-dimethylhydrazine (UDMH) in the Titan II missile system has made the determination of this compound in air, water, blood, and urine samples highly desirable. Several methods have been described for the determination of various hydrazine derivatives (refs. 1-5, 7-9), but none of these was specific for UDMH. The development of a screening procedure for urine was important since this laboratory has found that the urinary excretion of UDMH is an extremely sensitive and rapid indicator of exposure. In several preliminary experiments using animals that had received varying doses of UDMH, the ratio of the concentration per ml of urine to the concentration per ml of blood was approximately 20:1.

Experiments using monkeys (ref. 10) have indicated that pyridoxine hydrochloride in large doses (50 mg/kg*) is effective in preventing convulsions and death in animals receiving lethal doses of UDMH (100 mg/kg). Because of the discomfort associated with administration of such large therapeutic doses of pyridoxine (35 ml of a hypertonic solution for a 70-kg man), it would be advisable in an emergency situation to be guided by the urinary excretion level in the selection of cases which require treatment.

During the experimental phase of developing a procedure for analysis of urine, a factor inhibiting the formation of the color complex was observed in a large number of urines. Numerous attempts were made to eliminate the color-inhibiting substance(s).

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METHOD

Materials

Trisodium Pentacyanoamino Ferroate (TPF): Obtained from Fisher Scientific Co., Fairlawn, New Jersey, as Cat. No. S659. A 0.5 percent solution in distilled water is prepared daily. Since many investigators have reported lots of this reagent unsuitable using the procedure described in ASD TR 61-708 (ref. 6), the quality on each lot of this chemical must be checked by using the TPF evaluation technique described below. The TPF solution is stable for 8 hours at ordinary room temperature, but since TPF is a light-sensitive compound, the solution should be kept in a dark container.

In view of the difficulties with the Fisher reagent, sodium pentacyanoamino ferroate may be prepared in the laboratory:

Treat 10 grams of finely powdered (with mortar and pestle) sodium nitroprusside (Eastman Kodak - practical grade) in a flask with 32 ml of concentrated ammonium hydroxide overnight at refrigerator temperature.

Decant excess ammonium hydroxide and filter precipitate.

Wash the precipitate with absolute ethanol and anhydrous ether until dry.

The resulting sodium pentacyanoamino ferroate must be stored in a desiccator.

Buffer Solution: pH 5.4; 9.6 grams of citric acid (crystalline) and 15.7 grams of anhydrous disodium acid phosphate dissolved in 2 liters of distilled water.

Hydrogen Peroxide Solution: 0.3 percent; prepared from 3 percent H_2O_2 (USP) daily, using distilled water as the diluent. An accurate assay is required on long-standing solutions. A freshly opened bottle is preferable.

UDMH: 1,1-dimethylhydrazine (practical grade) obtained from Eastman Kodak Company, Rochester 3, New York.

UDMH Stock Standard Solution: Add 1.28 ml of UDMH to a 100-ml volumetric flask and dilute to the mark with distilled water. Allow the solution to cool before use. This solution contains 10 mg per ml and is usable for approximately 1 week.

UDMH Working Standard Solution: Transfer 1 ml of the stock standard solution to a 250-ml volumetric flask and dilute to the mark with distilled water. This solution contains 40 μg of UDMH per ml and must be freshly prepared.

Spectrophotometer: Coleman, Jr., Model 6A.

Cuvettes: 19 by 150 mm.

Procedure

Pipette 2 ml of urine to be tested into a cuvette.

Pool 5-ml aliquots of urine from 3 nonexposed persons, and pipette 2 ml of the pooled urine into another cuvette to serve as the blank.

Carefully pipette 0.02 ml of hydrogen peroxide into each cuvette. Wipe the outside of pipette before adding hydrogen peroxide and touch the drop to the inside of the tube just above the liquid level. Mix thoroughly by shaking.

Add 13 ml of buffer to each cuvette.

Add 1 ml of TPF reagent to each cuvette and mix.

Let the cuvettes stand for 20 minutes at room temperature to develop color.

Read the optical density of the unknown urine sample against the pooled negative urine blank set at 100 percent transmittance using 480 mμ.

Read concentration from the standardization curve.

If the optical density of the unknown exceeds the range of the standardization curve, repeat the test using an appropriate dilution of the urine.

Standardization Curve

Obtain 5 urine samples from nonexposed persons.

Set up 16 cuvettes (3 for each urine sample and 1 for a blank).

Pipette 2 ml of urine into 3 cuvettes from each specimen. Mark the first cuvette 10 μg, the second cuvette 20 μg, and the third cuvette 30 μg per ml.

Pipette 2 ml of a pooled specimen (equal volumes) from the 5 samples into another cuvette and mark it BLANK.

Add 0.5, 1.0, and 1.5 ml of the UDMH working standard solution to the respective cuvettes (all except the blank).

Carefully pipette 0.02 ml of hydrogen peroxide to each cuvette, including the blank. Wipe the outside of the pipette before delivering the H_2O_2 and touch the drop to the inside of the cuvette just above the liquid level. Mix by shaking.

Add sufficient buffer to bring the total volume in each cuvette to 15 ml.

Add 1 ml of TPF reagent to each cuvette and mix.

Let the cuvettes stand for 20 minutes at room temperature to develop color.

Read the optical densities of the calibration samples against the negative pooled urine blank set at 100 percent transmittance using 480 mμ.

Plot the mean values of the optical densities versus concentration of UDMH on linear graph paper for all 5 urines.

TPF Reagent Evaluation

This procedure is not part of the routine determination but must be used to determine the acceptability of each TPF lot.

Pipette 2 ml of buffer into 3 cuvettes.

Add 0.5, 1.0, and 1.5 ml of UDMH working standard solution to respective cuvettes.

Carefully pipette 0.02 ml of hydrogen peroxide solution to all cuvettes, observing the usual precautions. Mix by shaking.

Add sufficient buffer to bring the total volume to 15 ml.

Prepare a blank using 15 ml of buffer.

Add 1 ml of TPF reagent to all cuvettes. Mix.

Allow tubes to stand for 20 minutes.

Read the optical densities as previously described.

Construct calibration curve.

Refer to figure 1, calibration curve for TPF reagent.

If the angle of the slope of the constructed curve is substantially less than the curve in figure 1, the lot of TPF is not suitable for this test.

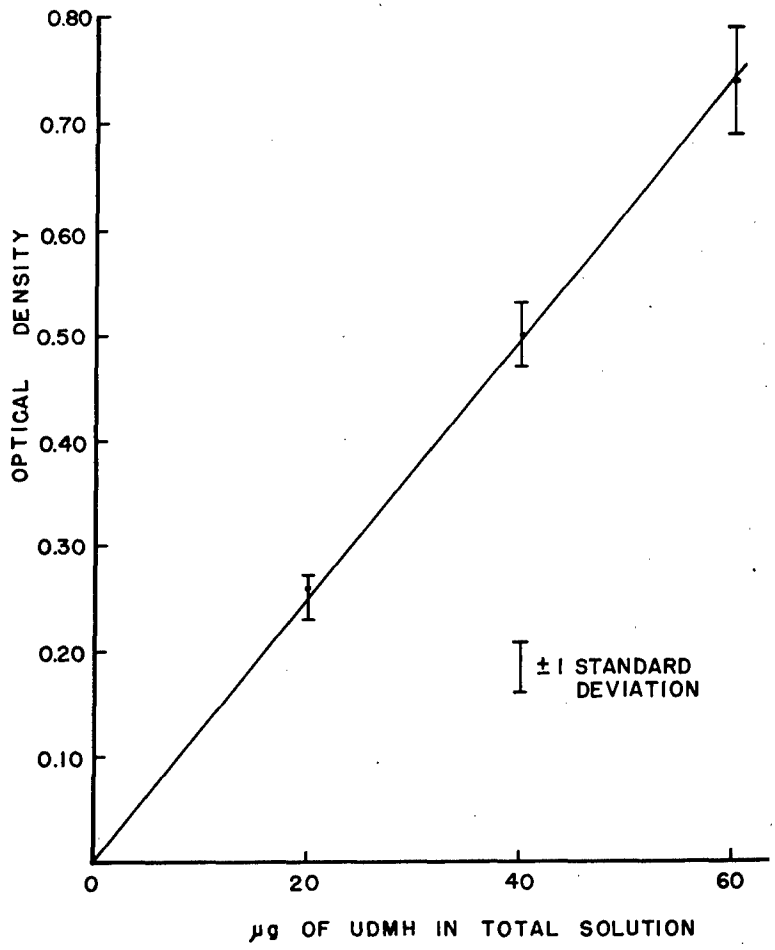


Figure 1. Calibration Curve for TPF Reagent

RESULTS

Effect of Hydrogen Peroxide on the Inhibitory Factor

The importance of using H_2O_2 is shown in figure 2, which compares results of the analyses of 20 random human urine specimens using the standardization curve procedure described above, with and without H_2O_2. The means and standard deviations of the optical densities for 10, 20, and 30 μg of UDMH per ml of urine, without H_2O_2, were 0.19 ± 0.07, 0.36 ± 0.10, and 0.53 ± 0.13. With H_2O_2, the comparative optical density values were 0.22 ± 0.05, 0.43 ± 0.05, and 0.63 ± 0.07. Although the difference does not appear to be significant, the trend toward greater recovery is quite apparent. The means of the optical densities were increased at every level of concentration when H_2O_2 was used.

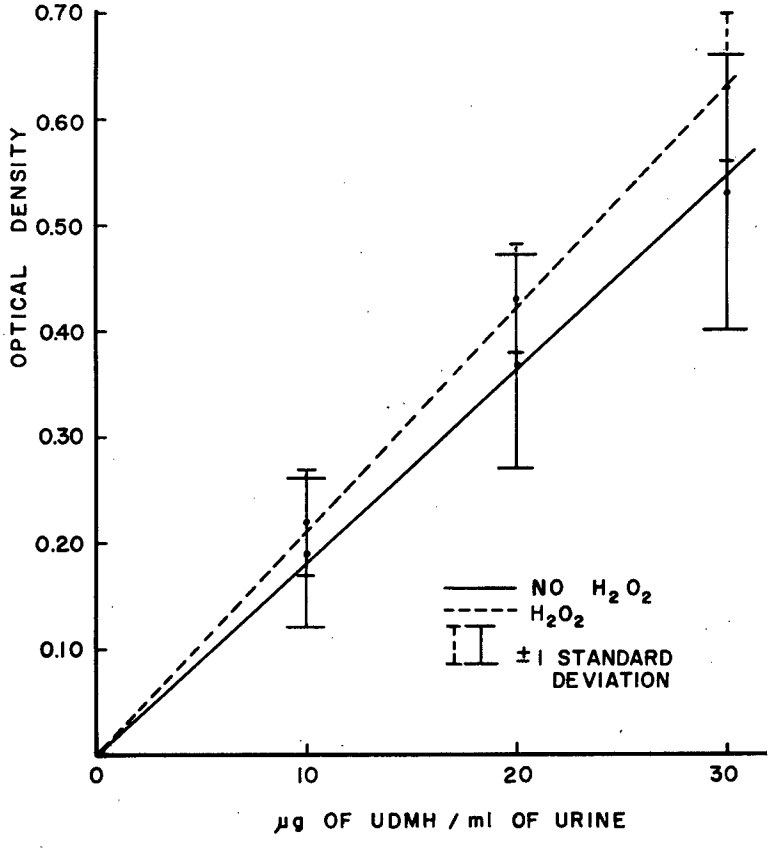


Figure 2. The Effect of H_2O_2 on Color Development

Table I illustrates this improvement much more clearly. Recovery in the individual urines that exhibited a high degree of inhibition without the addition of H_2O_2 was significantly improved. Such improvement is particularly apparent in the results obtained on specimens 5 and 7.

TABLE I

EFFECT OF HYDROGEN PEROXIDE ON RECOVERY OF UDMH FROM INDIVIDUAL URINE SAMPLES

(Expressed as optical density readings)

Specimen	Amount of Added UDMH to Each Urine Sample					
	10 μg/ml		20 μg/ml		30 μg/ml	
	Without H_2O_2	With H_2O_2	Without H_2O_2	With H_2O_2	Without H_2O_2	With H_2O_2
1	0.24	0.26	0.46	0.45		0.70
2	0.22	0.23	0.42	0.45	0.62	0.68
3	0.23	0.26	0.44	0.46	0.65	0.71
4	0.22	0.26	0.41	0.43	0.56	0.70
5	0.06	0.29	0.16	0.47	0.18	0.60
6	0.17	0.21	0.33	0.41	0.49	0.65
7	0.00	0.09	0.14	0.29	0.18	0.43
8	0.23	0.22	0.46	0.44	0.67	0.66
9	0.21	0.17	0.42	0.41	0.61	0.63
10	0.18	0.21	0.39	0.43	0.54	0.60
11	0.13	0.15	0.28	0.39	0.48	0.57
12	0.25	0.24	0.45	0.48	0.64	0.68
13	0.20	0.23	0.35	0.43	0.54	0.64
14	0.18	0.18	0.38	0.41	0.53	0.59
15	0.30	0.31	0.49	0.53	0.63	0.68
16	0.24	0.24	0.40	0.45	0.59	0.68
17	0.18	0.20	0.36	0.40	0.56	0.63
18	0.19	0.22	0.37	0.38	0.56	0.60
19	0.18	0.18	0.36	0.38	0.54	0.56
20	0.20	0.29	0.36	0.48	0.53	0.66
Averages	0.19	0.22	0.37	0.43	0.53	0.63
Standard Deviation	±0.07	±0.05	±0.10	±0.05	±0.13	±0.07

Recovery In Vivo

Five mongrel dogs were given water (30 mg/kg) by gastric intubation, and each was subjected to a single intraperitoneal injection of either 5, 10, 20, 30, or 40 mg/kg of UDMH. Following injection, they had access to water ad libitum. Urine samples were collected as they became available and analyzed for UDMH content using the method as described except for the addition of H_2O_2. The first few specimens were separately collected, and the final specimen was a pooled sample of all the urine subsequently produced by the animal within 24 hours. The results are listed in table II and indicate the relationship of dose to excretion level.

No valid correlation of dose with excretion level is possible from the results of such a limited number of animals. The data is presented merely to provide guidelines upon which to base an evaluation of human exposures. There appears to be a significant difference between urinary UDMH concentrations of the animal exposed to 10 mg/kg and of the one exposed to 40 mg/kg. Further, the relatively constant 24-hour excretion (as percentage of the total dose) in 3 out of 4 of the animals may well prove to be a valid figure when larger numbers of animals are used. The urine specimen from dog 3 that showed the least percent recovery in 24 hours (5.7 percent) may have contained the inhibitory agent which could have caused the low values obtained. Since this work was performed prior to the development of the H_2O_2 modification, we consider this explanation not only possible but probable.

TABLE II

UDMH URINARY EXCRETION IN DOGS

Dog	Weight (kg)	Dose (mg/kg)	Total Dose (mg)	Urine Sample Time after Injection	Concentration of UDMH (μg/ml)	Excreted Total of UDMH in Urine (mg)	Percent of Dose Excreted in 24 Hours
1	13.1	5	65.5	150 min 2.5-24 hr	43.0 8.7	6.2 1.8 8.0	12.2
2	6.8	10	68.0	15 min 45 min 0.75-24 hr	0.0 5.8 30.5	 0.4 7.7 8.1	11.9
3	10.7	20	214.0	120 min 180 min 3-24 hr	28.0 6.5 11.5	6.4 1.9 3.9 12.2	5.7
4	10.0	30	300.0	60 min 105 min 120 min 145 min 220-325 min 380 min	51.5 2.5 61.0 32.0 63.0 0.0	1.1 0.03 2.0 0.6 0.4 0.0 4.13	24-hr specimen lost
5	12.2	40	488.0	190 min 250 min 260 min 355 min 6-24 hr	360.0 40.0 130.0 160.0 59.0	1.1 0.4 1.6 33.3 18.5 54.9	11.3

Recovery In Vitro

Amounts of 10, 20, and 30 μg of UDMH per ml of urine were added to 100 human urines and the optical densities were obtained as described under Standardization Curve. The results are shown in figure 3. The means and standard deviations of optical densities for the above concentrations were, respectively: 0.22 ± 0.05, 0.43 ± 0.07, and 0.64 ± 0.09. With UDMH in buffer solution (figure 1), the means and standard deviations of the optical densities for the same concentrations of UDMH were 0.26 ± 0.02, 0.50 ± 0.03, and 0.74 ± 0.05. When compared with the recovery from buffer alone, the recovery of UDMH from buffered urine was 85 percent at the 10 μg/ml concentration, and 86 and 87 percent at the 20 and 30 μg/ml levels, respectively.

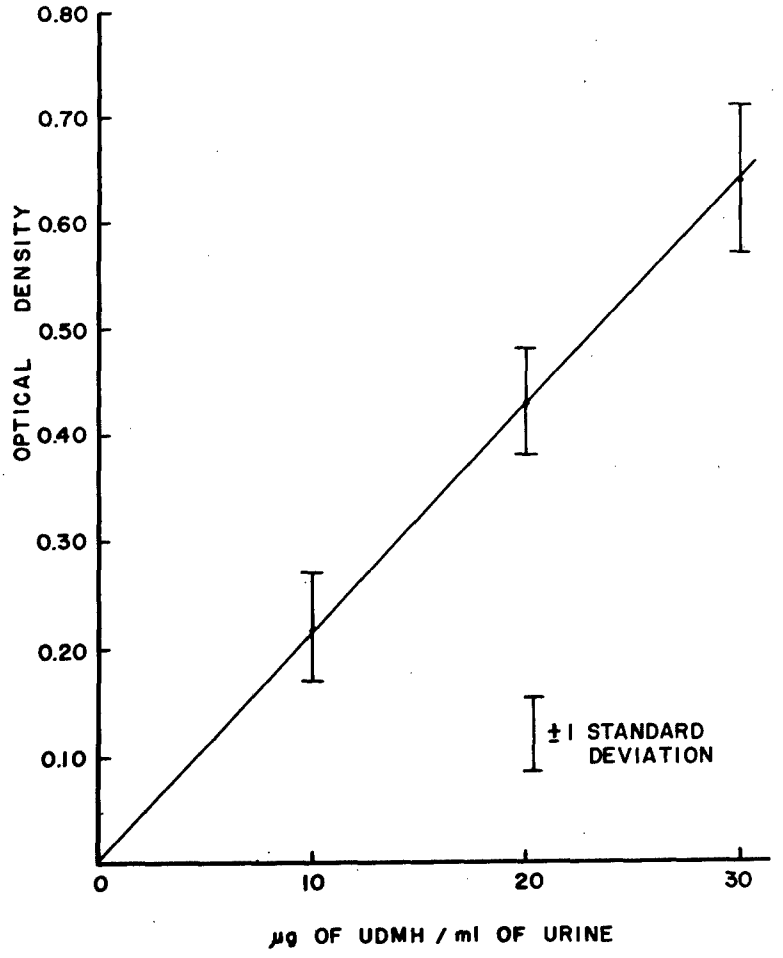


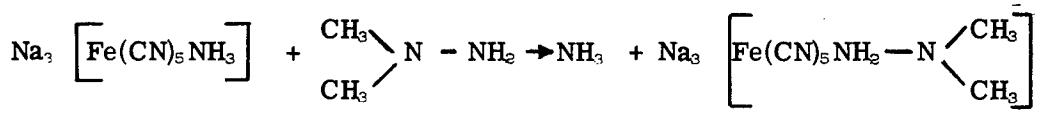
Figure 3. UDMH Recovery in vitro for 100 Urines

DISCUSSION

During the initial phase of this investigation, in vitro studies on the recovery of UDMH from human urine gave low erratic results due to the presence of a color-inhibiting substance(s) in urine which varied quantitatively from one specimen to another and appeared to be a commonly occurring constituent(s) of urine. Such unidentified color-inhibiting substance(s) presented the greatest problem in the development of a satisfactory and practical method. We observed no correlation between the extent of inhibition and the color of the urine. Complete inhibition of color was observed in some urine specimens even with the addition of 60 μg of UDMH per ml of urine. The potency of this factor was observed when as little as 0.4 ml of a severely inhibiting urine added to a 2-ml pooled urine specimen was capable of blocking color formation completely.

We attempted to adsorb both the UDMH and the color inhibitor from urine samples on activated Permutit columns, eluting only the UDMH with HCl. However, the tenacious adherence of UDMH to the column resulted in such poor recoveries that further efforts on a micro scale were considered impractical. Unsuccessful attempts were also made to extract the inhibitor with CCl_4 and $CHCl_3$, both with and without HCl treatment. Using low concentrations of NaOCl to selectively oxidize the inhibitor was tried and rejected.

We achieved partial success in overcoming the inhibition by using a more concentrated TPF solution than previously cited (ref. 6). Using H_2O_2 further enhanced recovery of UDMH. The exact mechanism of action of H_2O_2 on the inhibitor is not known at the present time. An irreversible reaction of H_2O_2 with the inhibitor-TPF complex appears to free the TPF for color formation with UDMH.* A soluble red color compound is formed with UDMH and TPF in urine, with an absorption optimum at 480 mμ. We assumed that this red color is the result of the replacement of the NH_3 molecule in the yellow ferroate by a molecule of UDMH as follows:



In approximately 250 UDMH-free urines no red color complex with TPF was observed during this test.

Chromogenic material, always present in urine, contributed to the somewhat large standard deviations in recovery studies. This variation was observed in an experiment in which the optical densities of 18 urines with added UDMH were corrected for their initial urine color. Since there is no need for great precision in a screening procedure, we felt that such correction was not essential.

In case of an extreme emergency involving significant exposure levels, an estimation of the final concentration of UDMH is possible almost immediately after the addition of TPF.

SUMMARY

A procedure for the determination of UDMH in urine was developed. The method includes treatment of urine with hydrogen peroxide and subsequent formation of a red TPF-UDMH complex. We attained 86 percent recovery for microgram quantities of UDMH in urine. There were no false negative results in over 100 urine specimens to which 10, 20, and 30 μg of UDMH per ml of urine were added. No false positive reactions were encountered. The procedure provides for rapid and convenient emergency determinations of significant UDMH concentrations in urine so that administration of effective therapy may be initiated before the onset of symptoms.

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Aerospace Medical Division, 6570th Aerospace Medical Research Laboratories, Wright-Patterson AFB, Ohio. Rpt. No. AMRL-TDR-62-119, DETERMINATION OF 1,1-DIMETHYLHYDRAZINE (UDMH) IN URINE. Final report, Oct 62, iii + 10 pp. incl. illus., tables, 10 refs.

Unclassified report

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1. UDMH
2. Methyl hydrazines
3. Ferrates
4. Urine
5. Colorimetric analysis

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